April 2010

MagAttract® RNA M48 Handbook

MagAttract RNA Cell Mini M48 Kit
For purification of total RNA from animal and human cells using the BioRobot® M48 workstation

MagAttract RNA Tissue Mini M48 Kit
For purification of total RNA from animal and human tissue using the BioRobot M48 workstation

MagAttract RNA Universal Tissue M48 Kit
For purification of total RNA from any type of animal or human tissue using the BioRobot M48 workstation

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"RNAlater™" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.

The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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QIAGEN Distributors **Error! Bookmark not defined.**

Kit Contents

MagAttract RNA M48 Kits	Cell Mini (192)	Tissue Mini (192)	Universal Tissue (192)
Catalog no.	**958236**	**959236**	**956336**
Number of preps	**192**	**192**	**192**
MagAttract Suspension E	2 x 13 ml	2 x 13 ml	2 x 13 ml
Buffer RLT*	2 x 45 ml	2 x 45 ml	–
Buffer MW*	3 x 50 ml	3 x 50 ml	4 x 50 ml
Buffer RPE†	2 x 55 ml	2 x 55 ml	2 x 55 ml
RNase-Free Water	1 x 90 ml	1 x 90 ml	1 x 90 ml
Buffer RDD (bottle)	2 x 35 ml	2 x 35 ml	–
DNase I, RNase-free (lyophilized)	2 x 1500 Kunitz units‡	2 x 1500 Kunitz units‡	–
Buffer RDD (tube)	8 x 2 ml	8 x 2 ml	–
RNase-Free Water	4 x 1.5 ml	4 x 1.5 ml	–
QIAzol Lysis Reagent*§	–	–	1 x 200 ml
Handbook	1	1	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 7 for safety information.

† Buffer RPE is supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

‡ Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. **33**, 349 and 363).

§ Packaged separately.

Additional QIAzol Lysis Reagent is available separately. The RNase-Free DNase Set is available separately for DNase digestion during the MagAttract RNA Universal Tissue procedure. See page 53 for ordering information.

Shipping and Storage

The MagAttract RNA Cell Mini M48 Kit and MagAttract RNA Tissue Mini M48 Kit are shipped at room temperature (15–25°C). The RNase-Free DNase Set box, containing RNase-free DNase I, Buffer RDD (tube), and RNase-free water, should

be stored immediately upon receipt at 2–8°C. The remaining components of the kit should be stored dry at room temperature. All kit components are stable for at least 9 months under these conditions.

The MagAttract RNA Universal Tissue M48 Kit is shipped at room temperature (15–25°C). QIAzol Lysis Reagent can be stored at room temperature or at 2–8°C, and is stable for at least 12 months under these conditions. The remaining components of the kit should be stored dry at room temperature, and are stable for at least 9 months under these conditions.

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of MagAttract RNA M48 Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

MagAttract RNA M48 Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of many of the materials described in this text. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding MagAttract RNA M48 Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer RLT, Buffer MW, and QIAzol Lysis Reagent contain guanidine thiocyanate or guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the BioRobot M48, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to the components of MagAttract RNA M48 Kits:

Buffer RLT

Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Buffer MW

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

The following risk and safety phrases apply to RNase-free DNase I, a component of the MagAttract RNA Cell Mini M48 Kit and the MagAttract RNA Tissue Mini M48 Kit:

RNase-free DNase I

Contains deoxyribonuclease: sensitizer. Risk and safety phrases:* R42/43, S22-24-26-36/37

The following risk and safety phrases apply to QIAzol Lysis Reagent, a component of the MagAttract RNA Universal Tissue M48 Kit.

QIAzol Lysis Reagent

Contains phenol, guanidine thiocyanate: toxic, corrosive. Risk and safety phrases:* R20-24/25-32-34, S13-26-36/37/39-45

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R20: Harmful by inhalation; R20/21/22: Harmful by inhalation, in contact with skin, and if swallowed; R22: Harmful if swallowed; R24/25: Toxic in contact with skin and if swallowed; R32: Contact with acids liberates very toxic gas; R34: Causes burns; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S22: Do not breathe dust; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible); S46: If swallowed, seek medical advice immediately and show this container or label.

Introduction

The MagAttract RNA Cell Mini M48 Kit is for purification of total RNA from animal and human cells. The MagAttract RNA Tissue Mini M48 Kit is for purification of total RNA from standard animal and human tissues. The MagAttract RNA Universal Tissue M48 Kit is for purification of total RNA from any type of animal and human tissue, or cultured cells.

MagAttract technology provides high-quality RNA that is suitable for direct use in downstream applications such as amplification or other enzymatic reactions. The BioRobot M48 performs all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 48 samples, in multiples of 6, are processed in a single run.

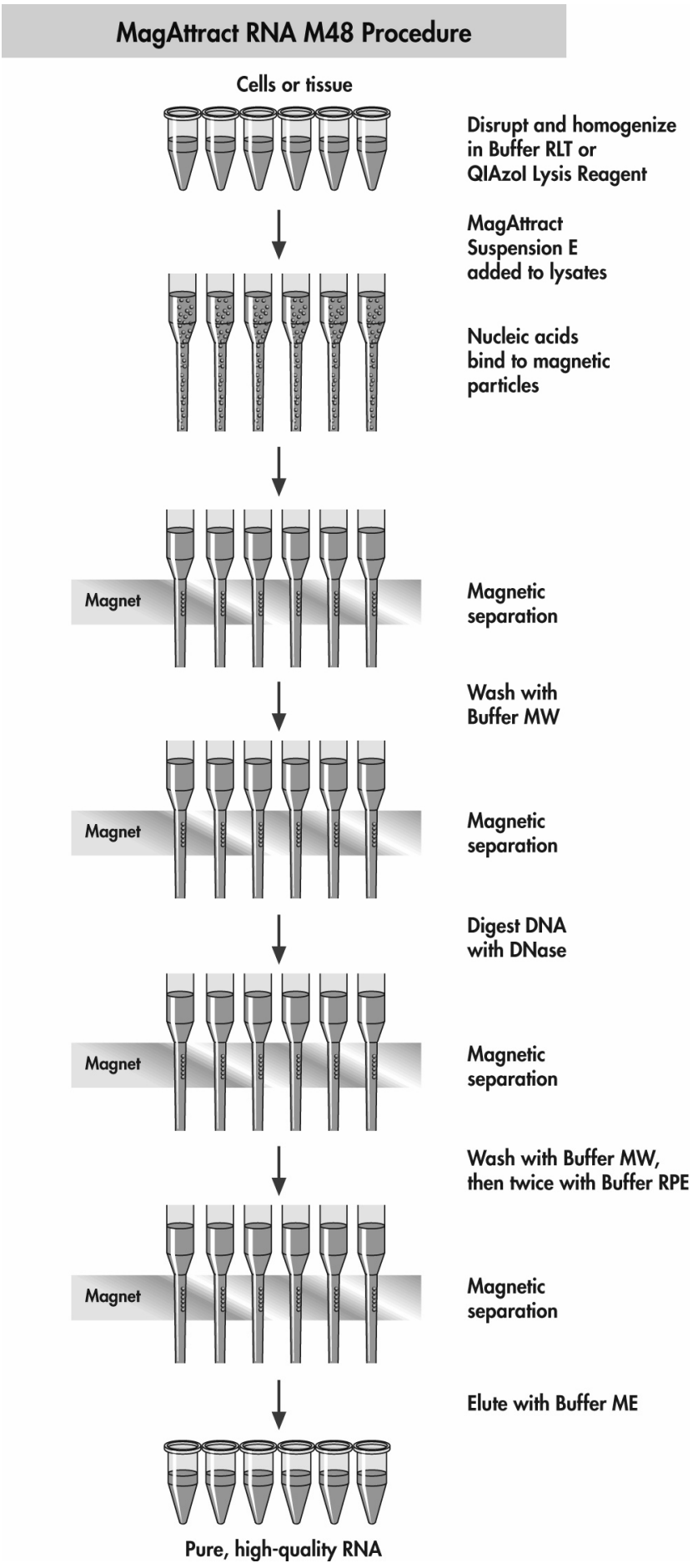
Principle and procedure

MagAttract technology combines the speed and efficiency of silica-based RNA purification with the convenient handling of magnetic particles. Nucleic acids are purified from lysates in one step through their binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet, and DNA is removed by treatment with RNase-free DNase.* Then, the magnetic particles are efficiently washed, and RNA is eluted in Buffer ME (see flowchart, page 10).

The MagAttract RNA Universal Tissue M48 Kit integrates efficient phenol/guanidine-based lysis and automated magnetic-particle purification. QIAzol Lysis Reagent, included in the kit, is a monophasic solution of phenol and guanidine thiocyanate, designed to inhibit RNases and to facilitate lysis of all types of tissue, including fatty and fiber-rich tissues. The high lysis efficiency of the reagent enables use of larger amounts of tissue (up to 50 mg of frozen tissue and up to 100 mg of adipose tissue).

Tissue samples are homogenized in QIAzol Lysis Reagent. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is extracted, and nucleic acids are purified in one step following the automated MagAttract RNA M48 procedure.

* When using the MagAttract RNA Universal Tissue M48 Kit, the optional DNase digestion requires the RNase-Free DNase Set (not supplied, see page 52 for ordering information).


MagAttract RNA M48 Procedure
Cells or tissue
Disrupt and homogenize in Buffer RLT or QIAzol Lysis Reagent
MagAttract Suspension E added to lysates
Nucleic acids bind to magnetic particles
Magnet
Magnetic separation
Wash with Buffer MW
Magnet
Magnetic separation
Digest DNA with DNase
Magnet
Magnetic separation
Wash with Buffer MW, then twice with Buffer RPE
Magnet
Magnetic separation
Elute with Buffer ME
Pure, high-quality RNA

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For all protocols

- BioRobot M48 workstation (workstations received before 1 January 2004 require updating with the M48 Software Upgrade Tool version 2.0, cat. no. 9016241)
- App. Package, M48, Gene Expression, cat. no. 9016149
- Sterile, RNase-free pipet tips
- Ethanol (96–100%)*
- Disposable gloves
- Equipment for disruption and homogenization (see pages 16–18)
- Filter-Tips, 1000 µl, M48 (1000), cat. no. 995652
- Reagent Containers, small, M48 (100), cat. no. 995902
- Reagent Containers, large, M48 (50), cat. no. 995904
- Reagent Container Seals, M48 (50), cat. no. 995906
- Sample Prep Plates, 42-well, M48 (100), cat. no. 995908
- Sample tubes, 1.5 ml, without lids (Sarstedt, cat. no. 72.696) or with screw caps (Sarstedt, cat. no. 72.692)†
- Optional: Sample tubes, 2 ml, without lids (Sarstedt, cat. no. 72.608) or with screw caps (Sarstedt, cat. no. 72.693)†
- Elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692) or 2 ml (Sarstedt, cat. no. 72.693)†

For total RNA purification from tissues using the MagAttract RNA Tissue Mini M48 Kit

- 14.3 M β-mercaptoethanol (β-ME)‡ (commercially available solutions are usually 14.3 M)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies; however, use of tubes other than those listed may result in an instrument crash.

‡ β-ME is added to Buffer RLT before use (see the protocols on pages 18 and 24).

For total RNA purification from cells or tissue using the MagAttract RNA Universal Tissue M48 Kit

- Chloroform (without added isoamyl alcohol)
- Microcentrifuge(s) (with rotor for 2 ml tubes) for centrifugation at 4°C, capable of attaining 12,000 x *g*
- Optional: RNase-Free DNase Set (cat. no. 79254)

Supplier of bead-mill homogenizers*

- QIAGEN TissueLyser system, comprising the TissueLyser, the TissueLyser Adapter Set 2 x 24, Stainless Steel Beads, 5 mm, and (optional) the TissueLyser Single-Bead Dispenser, 5 mm (see page 52 for ordering information)

Suppliers of rotor–stator homogenizers*

- Bio-Spec Products, Bartlesville, OK, USA (Tissue-Tearor™)
- Charles Ross & Son Company, Hauppauge, NY, USA
- Craven Laboratories, Austin, TX, USA
- IKA Analysentechnik GmbH, Germany (Ultra Turrax®)
- IKA Works, Cincinnati, OH, USA
- Kinematica AG; sold by Brinkmann Instruments, Westbury, NY, USA (Polytron® Homogenizers)
- OMNI International Inc., Waterbury, CT, USA (Omni Homogenizers)
- Silverson Machines, Bay Village, OH, USA
- Tekmar Inc., Cincinnati, OH, USA (Tissuemizer®)
- VirTis Company, Gardiner, NY, USA

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Determining the amount of starting material

The MagAttract RNA Cell Mini M48 Kit is optimized for RNA purification from up to 1 x 10^6 animal or human cultured cells, and from 10 to 2 x 10^6 human white blood cells. The MagAttract RNA Tissue Mini M48 Kit is optimized for RNA purification from up to 10 mg standard animal or human tissue, such as kidney, and from up to 5 mg of high-cell–density tissue such as spleen. The MagAttract RNA Universal Tissue M48 Kit is optimized for use with up to 1 x 10^7 animal or human cells or the amounts of animal or human tissue shown in Table 1. If you use more than these amounts, you may not achieve further increases in RNA yields.

Table 1. Amounts of Starting Material and Elution Volumes Used in MagAttract RNA M48 Procedures*

Sample	QIAsoft M Protocol	Amount of starting material	Elution volume
Cultured cells	MagAttract RNA Cell	≤1 x 10^6 cells†	50–200 µl
Cultured cells	MagAttract RNA Universal Tissue	≤1 x 10^7 cells†	50–200 µl
White blood cells	MagAttract RNA Cell	10 – 2 x 10^6 cells†	50–200 µl
Standard tissue‡	MagAttract RNA Tissue	1–10 mg†	50–200 µl
Standard tissue, flash-frozen‡	MagAttract RNA Universal Tissue	≤50 mg tissue§	50–200 µl
Adipose tissue	MagAttract RNA Universal Tissue	≤100 mg tissue§	50–200 µl
Liver, thymus, or spleen, flash-frozen	MagAttract RNA Universal Tissue	≤25 mg tissue§	50–200 µl
RNA*later*™ stabilized tissue	MagAttract RNA Universal Tissue	≤25 mg tissue§	50–200 µl

* Supplementary protocols (e.g., for purification of total nucleic acids) are available at www.qiagen.com/literature/clinlit.asp .

† Sample volume 400 µl.

‡ For example, kidney, lung, and intestine.

§ Sample volume 300–400 µl of upper aqueous phase after treatment with QIAzol Lysis Reagent and phase separation.

Direct counting is the most accurate way to quantify the number of cells. However, as a guide, the number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 2. When using the MagAttract RNA Cell Mini M48 Kit to purify total RNA from 1 x 10^6 HeLa cells, the average yield is 15 µg. RNA yield can vary due to species, developmental stage, growth conditions, etc.

Table 2. Growth Area and Number of HeLa Cells in Various Culture Dishes

Cell culture vessel	Growth area (cm^2)*	Number of cells†
Multiwell plates		
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x 10^6
Dishes		
35 mm	8	1 x 10^6
Flasks		
40–50 ml	25	3 x $10^{6‡}$

* Per well, if multiwell plates are used; varies slightly depending on the supplier.

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Numbers will vary for different kinds of animal cells, which vary in length from 10 to 30 µm.

‡ This number of cells exceeds the maximum binding capacity of the magnetic particles in the MagAttract RNA Cell Mini M48 procedure. To process this many cells, split the lysate into appropriate aliquots (≤1 x 10^6 cells each) and load them onto separate sample tubes.

Weighing is the most accurate way to quantify the amount of tissue. As a guide, a 1.5 mm cube (volume, approximately 3.4 mm^3) of most animal tissues weighs 3.5–4.5 mg. The average yield of total RNA varies depending on the type of tissue sample being processed. In addition, RNA yield can vary due to species, developmental stage, growth conditions, etc. When using the MagAttract RNA Tissue Mini M48 Kit to purify total RNA from 10 mg soft tissue, the average yield is 5–30 µg. Typical RNA yields using the MagAttract RNA Universal Tissue M48 Kit are given in Table 3 (page 15).

Table 3. Typical Total RNA Yields Using the MagAttract RNA Universal Tissue M48 Kit

Tissue	RNA yield (µg per 10 mg of tissue)*
Kidney	5–40
Liver	15–80
Lung	5–15
Heart	5–25
Muscle	5–35
Brain	5–20
Adipose tissue	0.5–2.5
Spleen	15–100
Intestine	10–60
Skin	2–5

* Amounts can vary due to species, age, gender, physiological state, etc.

Handling and storage of starting material

RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. See the *RNA*later *Handbook* for information about RNA*later* RNA Stabilization Reagent and about stabilizing RNA in tissues.

After harvesting or excision, samples can be immediately flash frozen in liquid nitrogen† and stored at –70°C. Frozen tissue should not be allowed to thaw during handling or weighing, but cell pellets can partially thaw enough to allow them to be dislodged by flicking. The relevant procedures should be carried out as quickly as possible.

Samples can also be stored at –70°C in lysis buffer (Buffer RLT or QIAzol Lysis Reagent) after disruption and homogenization. Frozen samples are stable for months.

† When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Disruption and homogenization of starting material

Efficient disruption and homogenization of the starting material is an absolute requirement at the start of the MagAttract RNA Cell Mini M48, MagAttract RNA Tissue Mini M48, and MagAttract RNA Universal Tissue M48 procedures. Disruption and homogenization are two distinct steps.

Disruption:	Complete disruption of plasma membranes of cells and organelles releases all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields.
Homogenization:	Homogenization reduces the viscosity of the cell lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the magnetic particles and therefore significantly reduced yields.

Some disruption methods simultaneously homogenize the sample while others require an additional homogenization step.

In the MagAttract RNA Cell Mini M48 procedure, disruption of cells is achieved by vortexing or mixing in Buffer RLT. The method of homogenization depends on the cell count of the sample. If the cell count is 1 x 10^5 cells or fewer, efficient homogenization is achieved by vortexing the sample. If the cell count is higher (1 x 10^5 to 1 x 10^6), homogenization must be performed using one of 4 methods:

- Rotor–stator homogenizer
- QIAshredder homogenizer
- Syringe and needle
- TissueLyser or other bead mill

In the MagAttract RNA Tissue Mini M48 procedure, disruption and homogenization of tissue can be performed using one of 3 methods:

- Disruption and homogenization using a rotor–stator homogenizer
- Disruption using a mortar and pestle, and homogenization using a QIAshredder homogenizer
- Disruption and homogenization using the TissueLyser or other bead mill

In the EZ1 RNA Universal Tissue procedure, disruption and homogenization of tissue can be performed using one of 2 methods:

- Disruption and homogenization using a rotor–stator homogenizer
- Disruption and homogenization using the TissueLyser or other bead mill

Note: After storage in RNA*later* RNA Stabilization Reagent, tissues become slightly harder than fresh or thawed tissues. Disruption and homogenization of this tissue, however, is usually not a problem.

The different disruption and homogenization methods are described in more detail below.

Disruption and homogenization using rotor–stator homogenizers

Rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize, in the presence of lysis buffer, cell lysates or tissues in 15–90 seconds depending on the toughness of the sample. The rotor turns at a very high speed and the combination of turbulence and mechanical shearing results in homogenization. Foaming of the sample should be kept to a minimum by using properly sized vessels, by keeping the tip of the homogenizer submerged, and by holding the immersed tip to one side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes. Probes with diameters of 5 mm and 7 mm are suitable for volumes of up to 300 µl and can be used for homogenization in microcentrifuge tubes. Probes with a diameter of 10 mm or above require larger tubes. See page 12 for a list of suppliers of rotor–stator homogenizers.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into a liquid-nitrogen–cooled, appropriately sized tube and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer and continue as quickly as possible with homogenization using the QIAshredder homogenizer (see below).

Note: Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization must be performed separately before proceeding with the MagAttract procedure.

Homogenization using QIAshredder homogenizers

Use of QIAshredder modules is a fast and efficient way to homogenize cell and tissue lysates without cross contamination of the samples. The lysate (volume of 400 µl for cell and tissue lysates) is loaded onto the QIAshredder spin column placed in a 2 ml collection tube, centrifuged for 2 minutes at maximum speed in a microcentrifuge, and the homogenized lysate collected. QIAshredder Spin Columns can be purchased separately for use with MagAttract Kits (see page 52 for ordering information).

Homogenization using a syringe and needle

Cell lysates can be homogenized using a syringe and needle. High-molecular-weight DNA can be sheared by passing the lysate through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may facilitate handling and minimize loss.

Disruption and homogenization using the TissueLyser system

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. Disruption efficiency is influenced by:

- size and composition of beads
- ratio of buffer to beads
- amount of starting material
- speed and configuration of agitator
- disintegration time

Stainless steel beads with a diameter of 5 mm are optimal to use for animal tissues in combination with the MagAttract RNA Tissue Mini M48 Kit or the MagAttract RNA Universal Tissue M48 Kit. All other disruption parameters should be determined empirically for each application.

The protocols for purification of total RNA from tissues (pages 24 and 30) contain guidelines for disruption and homogenization of tissues using the TissueLyser and stainless steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

Bead-milling can also be used to homogenize cell lysates. The optimal beads for use with animal or human cells are 3–7 mm diameter steel beads. The protocol for purification of total RNA from cells (page 19) contains guidelines for homogenization of cells using the TissueLyser and stainless steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

Quantification of RNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified RNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See Appendix B, page 43, for more information.

Protocol: Purification of Total RNA from Cells Using the MagAttract RNA Cell Mini M48 Kit

Important points before starting

- If using the MagAttract RNA Cell Mini M48 Kit for the first time, read "Important Notes" (page 13).
- If working with RNA for the first time, read Appendix A (page 41).
- If working with blood cells, read Appendix D (page 48).
- Cell pellets can be stored at –70°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that cell pellets can be dislodged by flicking in step 2. Homogenized cell lysates (in Buffer RLT, step 3) can be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature (15–25°C) or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. If any insoluble material is visible, centrifuge for 5 minutes at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.
- Buffer RLT and Buffer MW contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information. Take appropriate safety measures and wear gloves when handling.
- All steps of the protocol should be performed at room temperature (15–25°C).

Things to do before starting

- Before starting the procedure, ensure that MagAttract Suspension E is fully resuspended. Vortex for at least 3 min before first use, and for 1 min before subsequent uses.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature.

- β-ME may be optionally added to Buffer RLT before use to increase RNA yields. We do not recommend using β-ME except when purifying RNA from white blood cells (see Appendix D, page 48) or if RNA yields from previous purification procedures were low and the guidelines in the troubleshooting guide (page 35) have already been followed.

  If using β-ME, add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of β-ME.

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

- Prepare DNase I stock solution before using the RNase-free DNase I for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. **Do not vortex**.

  For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Harvest cells according to steps 1a (for cells grown in suspension) or 1b (for cells grown in a monolayer).

1a. Cells grown in suspension (do not use more than 1 x 10^6 cells): Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and continue with step 2 of the procedure.

Note: Incomplete removal of the cell-culture medium* will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

1b. Cells grown in a monolayer (do not use more than 1 x 10^6 cells):

Cells grown in a monolayer in cell-culture vessels can either be lysed directly in the culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet before lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

To lyse cells directly in culture dish:
Determine the number of cells. Completely aspirate the cell-culture medium,* and continue immediately with step 2 of the procedure.

Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

To trypsinize cells:
Determine the number of cells. Aspirate the medium, and wash cells with PBS.* Aspirate the PBS and add 0.10–0.25% trypsin* in PBS to trypsinize the cells. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and pellet by centrifugation at 300 x *g* for 5 min. Completely aspirate the supernatant, and continue with step 2 of the procedure.

Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

2. **Disrupt cells by addition of Buffer RLT. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 400 µl Buffer RLT. Vortex or pipet to mix, and proceed to step 3.**

   Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields.

   For direct lysis of cells grown in a monolayer, add 400 µl Buffer RLT to the cell-culture dish. Collect cell lysate with a rubber cell scraper. Pipet lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

3. **Homogenize the sample according to steps 3a, 3b, 3c, or 3d.**

   One of four methods may be used to homogenize the sample. See "Disruption and homogenization of starting material", page 16, for a more detailed description of homogenization methods.

   If $\leq 1 \times 10^5$ cells are processed, the cells can be homogenized by vortexing for 1 min.

   Note: Incomplete homogenization can affect binding of nucleic acids to the magnetic particles and lead to significantly reduced yields. Homogenization with rotor–stator or QIAshredder homogenizers generally results in higher RNA yields than with a syringe and needle.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

3a. Pipet the lysate directly onto a QIAshredder spin column (not supplied; see page 52 for ordering information) placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed. Continue the protocol with step 4.

3b. Homogenize cells for 30 s using a rotor–stator homogenizer. Continue the protocol with step 4.

3c. Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Continue the protocol with step 4.

3d. Transfer the lysate to a 2 ml microcentrifuge tube, and add one stainless steel bead (5 mm diameter). Homogenize the lysate for 2 min at 20 Hz using the TissueLyser. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant using a pipet. Continue the protocol with step 4.

Note: The instructions in step 3d are only guidelines. They may need to be changed depending on the cell sample being processed and on the bead mill being used.

4. Transfer the homogenized lysates to 1.5 ml or 2 ml sample tubes that are compatible with the sample rack of the BioRobot M48.

We recommend use of 1.5 ml sample tubes.

5. Ensure that the BioRobot M48 is switched on.

The power switch is on the left side of the instrument.

6. Switch on the computer and monitor.

7. Launch the QIAsoft M Operating System.

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop from the Microsoft® Windows® "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

8. Select the protocol group "Gene Expression" from the drop-down menu by clicking on the dark green arrow; select "Total RNA" and then "RNA Cell".

9. **Click the "Select" button to choose the elution tube type. Select the number of samples and the sample and elution volumes in the corresponding dialog fields. Click "Next".**

   The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol selected; these steps include the option of entering names for your samples. Be sure to follow all instructions that appear. Wear gloves when loading the required items on the worktable.

10. **Place the sample tubes on the workstation, plus reagent containers and plasticware, according to the software instructions.**

11. **Close the workstation door and start the protocol when instructed by the software. All subsequent steps are fully automated, and a software message on the screen will indicate when the protocol is finished.**

12. **Retrieve the elution tubes containing the purified RNA from the cooling block. The RNA is ready to use, or can be stored at –20°C or –70°C for longer periods.**

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to a clean tube (see Appendix B, page 43).

Protocol: Purification of Total RNA from Standard Tissues Using the MagAttract RNA Tissue Mini M48 Kit

Important points before starting

- If using the MagAttract RNA Tissue Mini M48 Kit for the first time, read "Important Notes" (page 13).
- If working with RNA for the first time, read Appendix A (page 41).
- For best results, stabilize animal tissues immediately in RNA*later* RNA Stabilization Reagent. Tissues can be stored in RNA*later* TissueProtect Tubes for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or for archival storage at –20°C or –80°C. See the *RNA*later *Handbook* for more information about RNA*later* RNA Stabilization Reagent and about stabilizing RNA in tissues.
- Fresh, frozen, or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen,* and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling before disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 3) can also be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 4.
- Buffer RLT and Buffer MW contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information. Take appropriate safety measures and wear gloves when handling.
- All steps of the protocol should be performed at room temperature (15–25°C).

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Things to do before starting

- Before starting the procedure, ensure that MagAttract Suspension E is fully resuspended. Vortex for at least 3 min before first use, and for 1 min before subsequent uses.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.
- Prepare DNase I stock solution before using the RNase-free DNase I for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. **Do not vortex**.

  For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. **Excise the tissue sample from the animal or remove it from storage. Remove RNA*later* stabilized tissues from the reagent using forceps. Do not use more than 10 mg tissue. Proceed immediately with step 2.**

   Weighing tissue is the most accurate way to determine the amount.

   Note: For tissues of high cell density, such as spleen, do not use more than 5 mg.

2. **Follow either step 2a or 2b, depending on how the tissues were stabilized.**

2a. For RNA*later* stabilized tissues:

If the entire piece of RNA*later* stabilized tissue can be used for RNA purification, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 3.

If only a portion of the RNA*later* stabilized tissue is to be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed with step 3.

RNA in the RNA*later* treated tissue is still protected while the tissue is processed at 18–25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent for further storage. Previously stabilized tissues can be stored at –80°C without the reagent.

2b. For unstabilized fresh or frozen tissues:

If the entire piece of tissue can be used for RNA purification, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately with step 3.

If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 3.

RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash frozen, or disrupted and homogenized in protocol step 3. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

Note: The remaining fresh tissue can be placed into RNA*later* RNA Stabilization Reagent for stabilization (see *RNA*later *Handbook*). However, previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

3. Disrupt tissue and homogenize lysate in Buffer RLT (do not use more than 10 mg tissue). Disruption and homogenization of animal tissue can be performed by 3 alternative methods (3a, 3b, or 3c).

See pages 16–18 for a more detailed description of disruption and homogenization methods.

After storage in RNA*later* RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem.

Note: Incomplete homogenization will lead to significantly reduced yields. Homogenization with rotor–stator homogenizers generally results in higher total RNA yields than with other homogenization methods.

3a. Rotor–stator homogenization:
Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a suitably sized vessel for the homogenizer. Add 400 µl Buffer RLT. Homogenize immediately using a conventional rotor–stator homogenizer until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 4.

Rotor–stator homogenization simultaneously disrupts and homogenizes the sample.

Note: For tissues of high cell density, such as spleen, use 500 µl of Buffer RLT.

3b. Mortar and pestle with QIAshredder homogenization:
Immediately place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add 400 µl Buffer RLT. Pipet the lysate directly onto a QIAshredder spin column placed in 2 ml collection tube, and centrifuge for 2 min at maximum speed. Continue the protocol with step 4.

Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization is carried out by centrifugation through the QIAshredder spin column.

Note: For tissues of high cell density, such as spleen, use 500 µl of Buffer RLT.

3c. TissueLyser homogenization:
Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a 2 ml microcentrifuge tube (not supplied), add 400 µl Buffer RLT, and add one stainless steel bead (3–7 mm diameter). Homogenize for 2 min at 20 Hz using the TissueLyser. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Continue the protocol with step 4.

Note: The instructions in step 3c are only guidelines. They may need to be changed depending on the sample being processed and on the bead mill being used.

Note: For tissues of high cell density, such as spleen, use 500 µl of Buffer RLT.

4. **Centrifuge the tissue lysate for 3 min at maximum speed in a microcentrifuge. Carefully transfer the supernatant to a new microcentrifuge tube (not supplied) by pipetting. Use only this supernatant (lysate) in subsequent steps.**

   In some preparations, very small amounts of insoluble material will be present, making the pellet invisible.

5. **Transfer the homogenized lysates to 1.5 ml or 2 ml sample tubes that are compatible with the sample rack of the BioRobot M48.**

   We recommend use of 1.5 ml sample tubes.

6. **Ensure that the BioRobot M48 is switched on.**

   The power switch is on the left side of the instrument.

7. **Switch on the computer and monitor.**

8. **Launch the QIAsoft M Operating System.**

   Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup window, but this setting may have been changed.

   The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop from the Microsoft Windows "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

9. **Select the protocol group "Gene Expression" from the drop-down menu by clicking on the dark green arrow; select "Total RNA" and then "RNA Tissue".**

10. **Click the "Select" button to choose the elution tube type. Select the number of samples and the sample and elution volumes in the corresponding dialog fields. Click "Next".**

    The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol selected; these steps include the option of entering names for your samples. Be sure to follow all instructions that appear. Wear gloves when loading the required items on the worktable.

11. **Place the sample tubes on the workstation, plus reagent containers and plasticware, according to the software instructions.**

12. **Close the workstation door and start the protocol when instructed by the software. All subsequent steps are fully automated, and a software message on the screen will indicate when the protocol is finished.**

13. **Retrieve the elution tubes containing the purified RNA from the cooling block. The RNA is ready to use, or can be stored at –20°C or –70°C for longer periods.**

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to a clean tube (see Appendix B, page 43).

Protocol: Purification of Total RNA from Any Type of Tissue or Cultured Cells Using the MagAttract RNA Universal Tissue M48 Kit

Important points before starting

- If using the MagAttract RNA Cell Mini M48 Kit for the first time, read "Important Notes" (page 13).
- If working with RNA for the first time, read Appendix A (page 41).
- For best results, stabilize animal tissues immediately in RNA*later* RNA Stabilization Reagent. Tissues can be stored in RNA*later* TissueProtect Tubes or RNA*later* for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or for archival storage at –20°C or –80°C. See the *RNA*later *Handbook* for more information about RNA*later* RNA Stabilization Reagent and about stabilizing RNA in tissues.
- Fresh, frozen, or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen,* and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling before disruption in QIAzol Lysis Reagents. Homogenized tissue lysates can also be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature (15–25°C) or at 37°C in a water bath until they are completely thawed and salts in the QIAzol Lysis Reagent are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 4.
- Generally, DNase digestion is not required since integrated QIAzol and MagAttract technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA remaining can be removed by using the automated protocol with an optional, integrated DNase digestion step or by DNase digestion after RNA purification (please contact QIAGEN Technical Services for a protocol).
- Buffer MW and QIAzol Lysis Reagent contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information. Take appropriate safety measures and wear gloves when handling.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- The centrifugation step to separate the aqueous from the organic phase (step 7) should be done at 4°C. All other steps of the protocol should be performed at room temperature.

Things to do before starting

- Before starting the procedure, ensure that MagAttract Suspension E is fully resuspended. Vortex for at least 3 min before first use, and for 1 min before subsequent uses.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

Procedure

1. **Excise the tissue sample from the animal or remove it from storage. Remove RNA*later* stabilized tissues from the reagent using forceps. Do not use more than 50 mg flash-frozen tissue, 25 mg liver, thymus, spleen, or RNA*later* stabilized tissue, or 100 mg adipose tissue. Proceed immediately with step 2.**

   Weighing tissue is the most accurate way to determine the amount.

2. **Follow either step 2a or 2b, depending on how the tissues were stabilized.**

2a. For RNA*later* stabilized tissues:

If the entire piece of RNA*later* stabilized tissue can be used for RNA purification, and place it directly into a 2 ml microcentrifuge tube (not supplied) for disruption and homogenization. Immediately pipet 750 µl QIAzol Lysis Reagent into each tube and proceed with homogenization.

If only a portion of the RNA*later* stabilized tissue is to be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it into a 2 ml microcentrifuge tube (not supplied) for homogenization. Immediately pipet 750 µl QIAzol Lysis Reagent into each tube.

RNA in the RNA*later* treated tissue is still protected while the tissue is processed at 18–25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent for further storage. Previously stabilized tissues can be stored at –80°C without the reagent.

2b. For unstabilized fresh or frozen tissues:
If the entire piece of tissue can be used for RNA purification, place it directly into a 2 ml microcentrifuge tube (not supplied) for disruption and homogenization. Immediately pipet 750 µl QIAzol Lysis Reagent into each tube and proceed with homogenization.

If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a 2 ml microcentrifuge tube (not supplied) for homogenization. Immediately pipet 750 µl QIAzol Lysis Reagent into each tube.

RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash frozen, or disrupted and homogenized in protocol step 3. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

Note: The remaining fresh tissue can be placed into RNA*later* RNA Stabilization Reagent for stabilization (see *RNA*later *Handbook*). However, previously frozen tissue samples thaw too slowly in the reagent, preventing fast diffusion of the reagent into the tissue before the RNA begins to degrade.

3. Disrupt tissue and homogenize lysate. Disruption and homogenization of animal tissue can be performed by 2 alternative methods (3a or 3b).

See pages 16–18 for a more detailed description of disruption and homogenization methods.

After storage in RNA*later* RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem.

Note: Incomplete homogenization will lead to significantly reduced yields. Homogenization with rotor–stator homogenizers generally results in higher total RNA yields than with other homogenization methods.

3a. Rotor–stator homogenization:
Homogenize using a conventional rotor–stator homogenizer until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 4.

Rotor–stator homogenization simultaneously disrupts and homogenizes the sample.

Some exceptionally tough tissues (e.g., human skin) may not be completely homogenized. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

3b. Bead mill homogenization:
Add one stainless steel bead (5 mm diameter) to each microcentrifuge tube containing tissue and QIAzol Lysis Reagent. Homogenize the lysates for 5 min at 25 Hz using the TissueLyser. Rotate the TissueLyser rack, and homogenize for another 5 min at 25 Hz. Continue the protocol with step 4.

Note: The instructions in step 3b are only guidelines. They may need to be changed depending on the sample being processed and on the bead mill being used.

Some exceptionally tough tissues (e.g., human skin) may not be completely homogenized. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

4. **Place the microcentrifuge tubes containing the homogenates on the benchtop at room temperature for 5 min.**
5. **Add 150 µl chloroform to each microcentrifuge tube containing the homogenized sample. Seal the microcentrifuge tubes securely, and shake them vigorously for 15 s.**

   Thorough mixing is important for subsequent phase separation.
6. **Place the microcentrifuge tubes on the benchtop at room temperature for 2–3 min.**
7. **Centrifuge the samples at 12,000 x *g* for 15 min at 4°C.**

   Centrifugation at 4°C is important for optimal phase separation and removal of genomic DNA.

   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high adipose content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µl.
8. **Transfer the upper aqueous phases (300–400 µl) to 1.5 ml or 2 ml sample tubes that are compatible with the sample rack of the BioRobot M48.**

   We recommend use of 1.5 ml sample tubes.
9. **Ensure that the BioRobot M48 is switched on.**

   The power switch is on the left side of the instrument.
10. **Switch on the computer and monitor.**

11. **Launch the QIAsoft M Operating System.**

    Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup window, but this setting may have been changed.

    The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop from the Microsoft Windows "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

12. **Select the protocol group "Gene Expression" from the drop-down menu by clicking on the dark green arrow; select "Total RNA" and then "Univ. Tissue".**

13. **Click the "Select" button to choose the elution tube type. Select the number of samples and the sample and elution volumes in the corresponding dialog fields. Click "Next".**

    The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol selected; these steps include the option of entering names for your samples. Be sure to follow all instructions that appear. Wear gloves when loading the required items on the worktable.

14. **Place the sample tubes on the workstation, plus reagent containers and plasticware, according to the software instructions.**

15. **Close the workstation door and start the protocol when instructed by the software. All subsequent steps are fully automated, and a software message on the screen will indicate when the protocol is finished.**

16. **Retrieve the elution tubes containing the purified RNA from the cooling block. The RNA is ready to use, or can be stored at –20°C or –70°C for longer periods.**

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to a clean tube (see Appendix B, page 43).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see back cover for contact information).

	Comments and suggestions
General handling	
QIAsoft M software error dialog box	If the QIAsoft M software displays an error dialog box during a protocol run, refer to the Troubleshooting Guide in the *BioRobot M48 User Manual*.
MagAttract RNA Universal Tissue M48 protocol: Phases do not separate completely	
a) No chloroform added or chloroform not pure	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
b) Homogenate not sufficiently mixed before centrifugation	After addition of chloroform (step 5), the homogenate must be vigorously shaken. If the phases are not well separated, shake the rack vigorously while inverting it for at least 15 s, and repeat the incubation and centrifugation (steps 6 and 7).
c) Organic solvents in samples used for purification	Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline reagents.* These can interfere with the phase separation.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

	Comments and suggestions
Low RNA yield	
a) Incomplete sample lysis	Before use, check that Buffer RLT does not contain a precipitate by shaking the bottle. Check again when pipetting Buffer RLT into a Reagent Container. If necessary, incubate for 30 min at 37°C with occasional shaking to dissolve precipitate.
b) MagAttract Suspension E was not completely resuspended	Before starting the procedure, ensure that MagAttract Suspension E is fully resuspended. Vortex for at least 3 min before first use, and for 1 min before subsequent uses.
c) Buffer RPE did not contain ethanol	Ensure that the correct volume of ethanol was added to Buffer RPE. Repeat the purification procedure with new samples.
d) Reagents were loaded onto worktable in wrong order	Ensure that all reagents were loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.
e) Insufficient disruption and homogenization	See "Disruption and homogenization of starting material" (pages 16–18) for a detailed description of homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see page 13 and protocol) and/or increase the volume of lysis buffer and the homogenization time.
f) Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 13 and protocol).

	Comments and suggestions
g) Incomplete removal of cell-culture medium	When processing cultured cells, ensure complete removal of the cell-culture medium after harvesting cells (see protocols).

RNA does not perform well in downstream applications

a) Insufficient RNA used in downstream application	Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm, (see "Quantification of RNA", Appendix B, page 43).
b) Excess RNA used in downstream application	Excess RNA can inhibit some enzymatic reactions. Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm, (see "Quantification of RNA", Appendix B, page 43).
c) Degraded RNA obtained from tissue samples	Too much sample may have been used. For most sample types, 10 mg tissue per 400 µl Buffer RLT is sufficient when using the MagAttract RNA Tissue M48 protocol. Larger amounts of tissue can be used with the MagAttract RNA Universal Tissue M48 protocol, as indicated in Table 1 on page 13.
d) Salt carryover during elution	Ensure that Buffer RPE is at 20–30°C.

A_{260}/A_{280} ratio for purified nucleic acids is low

a) Buffer RPE did not contain ethanol	Ensure that the correct volume of ethanol was added to Buffer RPE. Repeat the purification procedure with new samples.
b) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see "Quantification of RNA", page 43).

	Comments and suggestions
c) Wrong buffer used for RNA dilution	Use 10 mM Tris·Cl,* pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 43).
d) MagAttract RNA Universal Tissue M48 protocol: Not enough QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.
e) MagAttract RNA Universal Tissue M48 protocol: Sample not incubated for 5 min after homogenization	Place the sample at room temperature (15–25°C) for 5 min after homogenization, as indicated in the protocol.
RNA degraded	
a) Tissue sample not immediately stabilized	Submerge the sample in the appropriate volume of the RNA*later* RNA Stabilization Reagent immediately after harvesting the material.
b) Too much tissue sample for proper stabilization	Reduce the amount of starting material or increase the amount of RNA*later* RNA Stabilization Reagent used for stabilization (see *RNA*later *Handbook*).
c) Tissue sample too thick for stabilization	Cut large samples into slices less than 0.5 cm thick for stabilization in RNA*later* RNA Stabilization Reagent.
d) Frozen tissue samples used for stabilization	Use only fresh, unfrozen material for stabilization.
e) Storage duration exceeded	Storage of RNA*later* stabilized material is possible for up to 1 day at 37°C, up to 7 days at 18–25°C, and up to 4 weeks at 2–8°C. Store at –20°C or –80°C for archival storage.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

	Comments and suggestions
f) Sample inappropriately handled	For frozen cell pellets, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the protocol quickly, especially the first few steps. See Appendix A (page 41), and "Protocol: Purification of Total RNA from Cells" (page 19). Ensure that tissue samples are properly stabilized and stored in RNA*later* RNA Stabilization Reagent. For frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the protocol quickly, especially the first few steps. See Appendix A (page 41), and "Protocol: Purification of Total RNA from Standard Tissues Using the MagAttract RNA Tissue Mini M48 Kit" (page 24) or "Protocol: Purification of Total RNA from Any Type of Tissue or Cultured Cells Using the MagAttract RNA Universal Tissue M48 Kit" (page 30).
g) RNase contamination	Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 41). Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.
h) Tissue incubated in lysis buffer for too long before homogenization was started	After adding Buffer RLT or QIAzol Lysis Reagent to tissue, proceed with homogenization immediately.

	Comments and suggestions
MagAttract RNA Universal Tissue M48 protocol: DNA contamination in downstream experiments	
a) Phase separation performed at too high a temperature	The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
b) Interphase contamination of aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the eluate. Make sure to transfer the aqueous phase without interphase contamination.
c) No DNase treatment	Use the protocol with integrated DNase digestion using the RNase-Free DNase Set. Alternatively, after the MagAttract RNA Universal Tissue M48 procedure, DNase digest the eluate containing the RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment, or repurified using an RNA cleanup protocol.
Low reproducibility between samples	
a) Incomplete homogenization	Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample.
b) Variability between tissue samples	RNA yields from tissue samples can vary more than, for example, cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 42). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol*† and allowed to dry.

Solutions

Solutions (water and other solutions)* should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: The buffers of the MagAttract RNA Cell Mini M48 Kit, MagAttract RNA Tissue Mini M48 Kit, and MagAttract RNA Universal Tissue M48 Kit are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –20°C or –70°C in RNAase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260} = 1 → 44 µg/ml). This relation is valid only for measurements at neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity (see "Purity of RNA", page 44).

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH,* 1 mM EDTA* followed by washing with RNase-free water (see "Solutions", page 42). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the RNA in downstream applications. If it is necessary to minimize magnetic-particle carryover, the tube containing the eluate should first be placed in a suitable magnet (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912, for 1 minute) and the eluate transferred to a clean tube. If a suitable magnet is not available, centrifuge the tube containing the RNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.

When quantifying RNA samples, be sure also to measure the absorbance at 320 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

Concentration of RNA sample = 44 µg/ml x ($A_{260} – A_{320}$) x dilution factor

Total amount of RNA purified = concentration x volume of sample in ml

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl,† pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1‡ in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Quantification of RNA", page 43).

When determining the purity of RNA samples, be sure also to measure the absorbance at 320 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

Purity of RNA sample = $(A_{260} - A_{320}) / (A_{280} - A_{320})$

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. To prevent any interference by DNA in RT-PCR applications, we recommend working with intron-spanning primers so that genomic DNA will not be amplified. Alternatively, DNA contamination can be detected on agarose gels following RT-PCR by performing control experiments in which no reverse transcriptase is added before the PCR step. For sensitive applications, such as differential display, or if it is not practical to use intron-spanning primers, DNase digestion of the purified RNA with RNase-free DNase is recommended.

After the RNA purification procedure, the eluate containing the RNA can be treated with DNase. The RNA can then be repurified using an RNA cleanup protocol, or after heat inactivation of the DNase, the RNA can be used directly in downstream applications.

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

‡ Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

Integrity of RNA

The integrity and size distribution of total RNA purified with EZ1 RNA Kits can be checked by denaturing agarose* gel electrophoresis and ethidium bromide* staining (see "Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis", page 46) or using an Agilent 2100 bioanalyzer. The respective ribosomal RNAs (Table 4) should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S RNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

Table 4. Size of Ribosomal RNAs from Various Sources

Source	rRNA	Size (kb)
Mouse	18S	1.9
	28S	4.7
Human	18S	1.9
	28S	5.0

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

The following protocol for formaldehyde agarose (FA) gel electrophoresis is routinely used at QIAGEN and gives enhanced sensitivity for gel and subsequent analysis (e.g., northern blotting). A key feature is the concentrated RNA loading buffer that allows a larger volume of RNA sample to be loaded onto the gel than conventional protocols (e.g., Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

1.2% FA gel preparation

To prepare FA gel (1.2% agarose) of size 10 x 14 x 0.7 cm, mix:

1.2 g agarose*

10 ml 10x FA gel buffer* (see composition below)

Add RNase-free water to 100 ml

If smaller or larger gels are needed, adjust the quantities of components proportionately.

Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde* and 1 µl of a 10 mg/ml ethidium bromide* stock solution. Mix thoroughly and pour onto gel support. Before running the gel, equilibrate in 1x FA gel running buffer (see composition on next page) for at least 30 minutes.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x loading buffer (see composition on next page) per 4 volumes of RNA sample (for example 10 µl of loading buffer and 40 µl of RNA) and mix.

Incubate for 3–5 minutes at 65°C, chill on ice, and load onto the equilibrated FA gel.

Gel running conditions

Run gel at 5–7 V/cm in 1x FA gel running buffer.*

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Composition of FA gel buffers

10x FA gel buffer

200 mM 3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)*

50 mM sodium acetate*

10 mM EDTA*

pH to 7.0 with NaOH*

1x FA gel running buffer

100 ml 10x FA gel buffer

20 ml 37% (12.3 M) formaldehyde

880 ml RNase-free water

5x RNA loading buffer

16 µl saturated aqueous bromophenol blue solution*†

80 µl 500 mM EDTA, pH 8.0

720 µl 37% (12.3 M) formaldehyde

2 ml 100% glycerol*

3084 µl formamide*

4 ml 10x FA gel buffer

RNase-free water to 10 ml

Stability: Approximately 3 months at 4°C

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† To make a saturated solution, add solid bromophenol blue to distilled water. Mix and continue to add more bromophenol blue until no more will dissolve. Centrifuge to pellet the undissolved powder, and carefully pipet the saturated supernatant.

Appendix D: Preparing Human Blood Cells for Purification of Total RNA

The protocol for purification of total RNA from cells (page 19) can be used to purify total RNA from human blood cells. This appendix contains information on how to prepare blood cells for this protocol. Please read this information before starting the protocol.

Collecting, storing, and handling samples

The MagAttract RNA Cell Mini M48 Kit is suitable for purification of total cellular RNA from fresh, whole human blood. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA,* although other anticoagulants such as citrate,* heparin,* or ACD (acid citrate dextrose)* can also be used.

For optimal results, blood samples should be processed within a few hours of collection. mRNAs from blood cells have different stabilities. mRNAs of regulatory genes have shorter half-lives than mRNAs of housekeeping genes. To ensure that the purified RNA contains a representative distribution of mRNAs, blood samples should not be stored for long periods before purifying RNA.

Note: The MagAttract RNA Cell Mini M48 Kit cannot be used for frozen blood samples.

Starting amounts of samples

A maximum amount of 500 µl of whole blood from healthy adults (typically 4000–7000 leukocytes per microliter) can be processed per sample tube using the MagAttract RNA Cell Mini M48 Kit. For blood with elevated numbers of leukocytes, less than 500 µl must be used. The maximum number of leukocytes that can be processed is 2×10^6 per sample tube. If more leukocytes are processed, they will not be fully lysed and contaminants will not be completely removed.

Maximum RNA yields using the MagAttract RNA Cell Mini M48 Kit are generally determined by two criteria: lysis volume and binding capacity of the magnetic particles. Using the maximum amount of leukocytes that can be processed in the procedure (2×10^6), however, the binding capacity of the magnetic particles is not usually attained due to the low RNA content of leukocytes.

Lysis and homogenization

Blood cells are lysed in two separate steps, erythrocyte lysis and leukocyte lysis. Erythrocytes (red blood cells) of human blood do not contain nuclei and are

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

therefore not important for RNA purification since they neither synthesize nor contain RNA. The target of RNA purification from whole blood is leukocytes (white blood cells), which are nucleated and therefore do contain RNA. Leukocytes consist of 3 main cell types: lymphocytes, monocytes, and granulocytes.

Erythrocyte lysis

Since healthy blood contains approximately 1000 times more erythrocytes than leukocytes, removing the erythrocytes simplifies RNA purification. The procedure described in this appendix (see below) uses selective lysis of erythrocytes to achieve this. Erythrocytes are more susceptible than leukocytes to hypotonic shock and burst rapidly in the presence of a hypotonic buffer.

Intact leukocytes are then recovered by centrifugation. The conditions for selective lysis of erythrocytes in the procedure below have been optimized to allow fast removal of erythrocytes without affecting the stability of the leukocytes. The erythrocyte-lysis step can be scaled up for volumes of whole blood >50 µl.

A common alternative to erythrocyte lysis is Ficoll® density-gradient centrifugation. In contrast to erythrocyte lysis, Ficoll density-gradient centrifugation only recovers mononuclear cells (lymphocytes and monocytes) and removes granulocytes. Mononuclear cells isolated by Ficoll density-gradient centrifugation can be processed using the MagAttract RNA Cell Mini M48 Kit.

Both erythrocyte lysis and Ficoll density-centrifugation rely upon intact blood cells, so fresh blood must be used.

Leukocyte lysis

During the MagAttract RNA Cell Mini M48 procedure, leukocytes are efficiently lysed under highly denaturing conditions that immediately inactivate RNases, allowing purification of intact RNA.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- **Recommended**: Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of β-ME.

Procedure

D1. Mix 1 volume of whole human blood with 5 volumes of Buffer EL (see ordering information, page 53) in an appropriately sized tube (not supplied).

For optimal results, the volume of the mixture (blood + Buffer EL) should not exceed ¾ of the volume of the tube to allow efficient mixing. For example, add 1000 µl of Buffer EL to 200 µl of whole blood, and mix in a tube which has a total volume of ≥1600 µl.

Note: Use an appropriate amount of whole blood. Up to 500 µl of healthy blood (typically 4000–7000 leukocytes per microliter) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used.

D2. Incubate for 10–15 min on ice. Mix by vortexing briefly 2 times during incubation.

The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 min.

D3. Centrifuge at 400 x *g* for 10 min at 4°C, and completely remove and discard supernatant.

Leukocytes will form a white pellet after centrifugation. Ensure supernatant is completely removed. Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in step D4.

However, if erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5–10 min on ice after addition of Buffer EL in step D4.

D4. Add Buffer EL to the cell pellet (use 2 volumes of Buffer EL per volume of whole blood used in step D1). Resuspend cells by vortexing briefly.

For example, add 400 µl of Buffer EL per 200 µl of whole blood used in step D1.

D5. Count the cells, and transfer a volume of cell suspension that corresponds to 2 x 10^6 cells to a microcentrifuge tube. Centrifuge at 400 x *g* for 10 min at 4°C, and completely remove and discard supernatant.

Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the magnetic particles, resulting in lower yield.

D6. Continue with step 2 of the protocol for purification of total RNA from cells (page 21).

Ordering Information

Product	Contents	Cat. no.
MagAttract RNA Cell Mini M48 Kit (192)	MagAttract Suspension E and buffers for up to 192 preps	958236
MagAttract RNA Tissue Mini M48 Kit (192)	MagAttract Suspension E and buffers for up to 192 preps	959236
MagAttract RNA Universal Tissue M48 Kit (192)	MagAttract Suspension E, QIAzol Lysis Reagent, and buffers for up to 192 preps	956336
Accessories		
BioRobot M48	Robotic workstation for automation of magnetic-particle purification technology	9000708
App. Package, M48, Gene Expression	Software protocol package for gene expression applications on the BioRobot M48 workstation	9016149
Starter Pack, M48	Pack includes: sterile filter-tips (600); sample prep plates (40); large reagent containers (8); small reagent containers (8); silicon seals (8); sample tubes, 1.5 ml (250); sample tubes, 2 ml (250); elution tubes, screw cap, 1.5 ml (250); tip waste bags (2)	995999
Filter-Tips, 1000 µl, M48 (1000)	Sterile, disposable filter-tips, bagged; pack of 1000	995652
Reagent Containers, small, M48 (100)	Reagent containers (20 ml) with lids. To be used with the Reagent Container Rack, M48; pack of 100.	995902

Product	Contents	Cat. no.
Reagent Containers, large, M48 (50)	Reagent containers (110 ml) with lids. To be used with the Reagent Container Rack, M48; pack of 50.	995904
Reagent Container Seals, M48 (50)	Lid-sealing sheets for small and large reagent containers, allowing storage of unused reagents; pack of 50	995906
Sample Prep Plates, 42-well, M48 (100)	Disposable polypropylene plates for sample preparation, including nucleic acid binding and washing steps; pack of 100	995908
Cooling Block, 48-tube, 0.2 ml, M48	Holder for accommodating 48 x 0.2 ml PCR tubes on the cooling and heating system of the BioRobot M48 worktable	9015178
Cooling Block, 48-tube, 1.4 ml, M48	Plastic holder for accommodating 1.4 ml tubes on the cooling and heating ysystem of the BioRobot M48 worktable	9015180
QIAshredder (50)	50 disposable cell-lysate homogenizers, caps	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers, caps	79656
TissueLyser	Universal laboratory mixer-mill disruptor	Inquire
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser	69982
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965

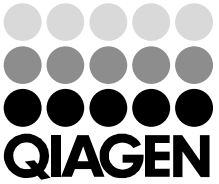
Product	Contents	Cat. no.
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254
RNA*later* RNA Stabilization Reagent (50 ml)	50 ml RNA*later* RNA Stabilization Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNA*later* RNA Stabilization Reagent (250 ml)	250 ml RNA*later* RNA Stabilization Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106
RNA*later* TissueProtect Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA*later* RNA Stabilization Reagent each	76154
RNA*later* TissueProtect Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNA*later* RNA Stabilization Reagent each	76163
QIAzol Lysis Reagent (200 ml)	200 ml QIAzol Lysis Reagent	79306
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254
Buffer EL (1000 ml)	1000 ml Erythrocyte Lysis Buffer	79217

Product	Contents	Cat. no.
Related products		
MagAttract Direct mRNA M48 Kit (192)	MagAttract Suspension C and buffers for up to 192 preps	957236
MagAttract DNA Blood Mini M48 Kit (192)	MagAttract Suspension B and buffers for up to 192 preps	951336
MagAttract DNA Blood Midi M48 Kit (192)	MagAttract Suspension B and buffers for up to 192 preps	951356
MagAttract DNA Mini M48 Kit (192)	MagAttract Suspension B and buffers for up to 192 preps	953336

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes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